International Journal of Research in Medical Sciences
Naz N et al. Int J Res Med Sci. 2019 Aug;7(8):2985-2988
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012

DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20193382

Original Research Article

Diagnosis of tubercular lymphadenopathy by fine needle aspiration cytology and Z-N staining

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Received: 14 March 2019
Revised: 30 June 2019
Accepted: 10 July 2019

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ABSTRACT

Background: Tuberculosis continues to be the biggest health problem in India. Tuberculosis involves respiratory, gastrointestinal tract as well as extrapulmonary site. Tubercular lymphadenopathy is the most common form of extrapulmonary tuberculosis. FNAC plays a vital role in diagnosis of tubercular lymphadenopathy. FNAC is not only used for cytological diagnosis but also used for other ancillary tests like Ziehl-Neelsen staining and AFB culture.
Methods: The study was conducted in the department of pathology, Government Medical College, Jammu over a period of 6 months and included 450 cases presenting with superficial lymphadenopathy. FNAC was performed in the cases and smears in each case, were stained with May Grunwald Giemsa (MGG), Papanicolaou and Z-N stain.
Results: Out of 450 cases,160 cases (35.5%) showed features of tubercular lymphadenitis. The most common site of presentation, being cervical region with 75% cases. Females outnumbered males by ratio of 1.46:1. In cytomorphology 93 cases (58.1%) showed epithelioid granulomas with caseous necrosis,37 cases (23.1%) showed caseous necrosis only while only epithelioid granulomas were seen in 30 cases (23.1%). AFB positivity was seen in 82 cases with maximum AFB positivity (78.3%) seen in cases with necrosis only.
Conclusions: FNAC is a rapid diagnostic technique because of its simplicity, cost effectiveness, early availability of results and minimal invasion. FNAC is a reliable and sensitive first line investigation in diagnosis of tubercular lymphadenitis combined with AFB staining.

Keywords: Granulomas, Lymphadenopathy, Necrosis, Tuberculosis

INTRODUCTION

Tuberculosis continues to be the biggest health problem in developing country. India has the highest burden of tuberculosis in the world and accounts for approximately one-fifth of the global incidence.[1] Tuberculosis involves respiratory, gastrointestinal tract as well as extrapulmonary site. Tubercular lymphadenopathy is the most common form of extrapulmonary tuberculosis and constitutes app 20-40% of extrapulmonary tuberculosis.[2]

FNAC plays a vital role and is recognized as a rapid diagnostic technique because of its simplicity, cost effectiveness, early availability of results and minimal invasion. In tubercular lymphadenopathy, FNAC is not only used for cytological diagnosis but also used for other ancillary tests like Ziehl-Neelsen staining and AFB culture. Standard diagnostic algorithm for TBLN in India recommends FNAC along with Ziehl-Neelsen (ZN) staining for acid fast bacilli (AFB) in clinically suspected patients.[3] The aim of study was to determine the utility of

fine needle aspiration in diagnosis of Tuberculosis and correlation of Z -N stain (AFB positivity).

METHODS

The present prospective study carried out in the department of Pathology, Government Medical College, Jammu, Jammu and Kashmir, India over a period of 6 months from August 2018 to Jan 2019 and included 450 cases of superficial lymphadenopathy who were referred for FNA cytology. In all these cases, FNAC was performed under aseptic conditions, using 23 G needle with or without aspiration. Minimum of three stains May Grunwald Giemsa (MGG), Papanicolaou and Z-N stain were done in each case.

Pap staining

PAP is a polychromatic stain containing multiple dyes to differentially stain various components of the cells. Pap staining is used to differentiate cells in smear preparations from fine needle aspirations. Pap stain involves five dyes in 3 solutions-Harris haematoxyl in, Orange Green-6 (OG 6) and eosin azure. For PAP stain: Smears were immediately fixed in 95% alcohol for 5-15 mins. Do not allow smears to dry.

May grunwald giemsa staining

MGG is a type of Romanowsky stain, it contains both acidic and basic dyes which have affinity for acidic and basic components of the cells resp. It is used to differentiate nuclear and cytoplasmic morphology of the cells. For MGG stain, fixation is not required. The smear is air dried and then stained.

Ziehl-Neelsen staining

Z-N staining is done to identify acid fast bacilli *esp mycobacterium*. *Mycobacterium* contain high conc of lipid making them hydrophobic and impermeable to routine stain such as gram stain. They are also resistant to acid and alcohol. In Z-N staining they are stained with carbol fuchsin combined with phenol and heated to enable the dye to penetrate the mycobacterial cell wall. After staining, an acid decolorizing solution is applied. This removes the red dye from background except *mycobacteria* which retain the dye and are therefore referred to as acid fast bacilli. Record was made of all the relevant findings age, sex, site, size and character of the aspirate.

Inclusion criteria

Only peripheral superficial lymphadenopathies cases were included.

Exclusion criteria

Deep and abdominal lymphadenopathies were excluded.

RESULTS

A total of 450 cases of superficial lymphadenopathies were aspirated. Out of 450 cases, 160 cases (35.5%) showed features of Tubercular lymphadenitis.

Reactive lymphoid hyperplasia was seen in 196 cases (43.5%). Suppurative lymphadenitis, metastatic carcinoma and lymphomas were seen in 36, 40 and 18 cases respectively (Table 1).

Table 1: Distribution of cases.

Diagnosis	Cases
Reactive lymphoid hyperplasia	196 (43.5%)
Tubercular lymphadenitis	160 (35.5%)
Suppurative lymphadenitis	36 (8%)
Metastatic carcinoma	40 (9%)
Lymphomas	18 (4%)
Total	450

The most common site for aspiration was cervical lymphandenopathy with 75% cases, followed in frequency by axillary (20%), inguinal (4%) and generalized (1%) lymphandenopathy.

In this study, the age of the patients ranged from 6 months to 74 years with maximum no of cases seen in 3rd (46 cases) and 4th (37 cases) decade of life, followed by 11-20 age group with 25 cases.

Next in frequency was 41-50 age group (21 cases). Least no of cases were seen among >60 years of age.

In the present study, female's predominance was observed especially in 2nd and 3rd decade. In 31-40 age group also, female outnumbered males, while in rest of the age groups males were slightly on the high side as compared to females (Table 2).

Table 2: Age wise and sex wise distribution of cases of tubercular lymphadenitis.

Age group	Males	Females	Total
0-10 years	9	7	16
11-20 years	9	16	25
21-30 years	7	30	37
31-40 years	17	29	46
41-50 years	13	8	21
51-60 years	7	3	10
>60 years	3	2	5
Total	65	95	160

Overall, females outnumbered males by a ratio of 1.46:1. In cytomorphology, 93 cases (58.1%) showed epithelioid granulomas along with caseous necrosis, 37 cases (23.1%) showed caseous necrosis only while only epithelioid granulomas were seen in 30 cases (23.1%).

Overall 82 cases showed AFB positivity and out of this maximum AFB positivity of smears was seen in cases with necrosis only i.e. 78.3% cases and least (6.6%) in cases with epithelioid granulomas only (Table 3).

Table 3: Cytomorphological pattern and correlation with AFB stain.

Features	Cases	AFB positivity
Epithelioid granulomas with caseous necrosis	93 (58.1%)	51 (54.8%)
Epithelioid granulomas without caseous necrosis	30 (18.8%)	2 (6.6%)
Caseous necrosis only	37 (23.1%)	29 (78.3%)

DISCUSSION

TB, a contagious disease caused by Mycobacterium TB, affects respiratory tract as well as extrapulmonary sites. Tuberculous lymphadenitis is the most common form of extrapulmonary TB in regions with high prevalence of mycobacterial infection.[4] FNAC is simple, non-invasion tool with high sensitivity in diagnosing tuberculosis esp in developing countries.

In this study, out of the total 450 cases of lymph nodes aspirates referred to the cytological section,160 smears show cytomorphological features of tubercular lymphadenitis, the incidence being 35.5%. This institution is a tertiary care referral center catering to a large population, thus justifying high incidence. Ahmad et al, reported incidence of 38%. Gupta et al, and Tilak et al, reported 34.6% and 38.8% incidence resp.[5-7]

In this study, the age range of patients was from 6 months to 74 years with majority of patients (51.8%) in 2nd and 3rd decade of life. Similar pattern was seen in study by Mahapatra who reported maximum patients in 2nd decade followed by 3rd decade. Other concordant studies were reported by Gupta et al, Paliwal et al, and Ergrte et al.[6,8-10]

In the present study, females (95 cases) outnumbered males (65 case) by the ratio of 1.46:1. This is in agreement with study by Fatima et al, where also female preponderance was reported. However, Ahmad et al, and Rajsekharan et al, reported higher incidence in males.[5,11,12]

In the present study, cervical region was the most commonly affected region involved in 75% of the cases. This in is agreement with Bezabith et al, who also observed cervical involvement in 74.2% of the cases.[13]

In this study, the most cytological pattern was epitheliod granulomas with caseous necrosis in 58.1% case followed by necrosis only in 23.1% cases. This was similar to studies by Gupta et al, and Chand et al, However, studies by Nidhi et al, and Paliwal et al, reported presence of necrosis as the most common pattern.[6,9,14,15]

The overall AFB positivity this study was 51.2%with the highest AFB positivity seen in smears with necrosis only (78.3%).

Mitra et al, reported overall AFB positivity of 51.6%, out of which the highest positivity was seen in smears revealing necrosis only with or without epithelioid cell granulomas (78.1%).[16] Gupta et al, reported overall AFB positivity of 65% with maximum positivity (75%) in necrosis with polymorphs and with or without epithelioid granulomas, while Mistry et al, Dua et al, and Aggarwal et al, reported low AFB positivity of 22.9%, 27.1% and 1 9.6% resp.[4,6,17,18]

CONCLUSION

FNAC has proved to be a reliable and sensitive first line investigation in diagnosis of tubercular lymphadenitis. The accuracy can be further enhanced by using AFB staining.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Naz N, Sharma M. Diagnosis of tubercular lymphadenopathy by fine needle aspiration cytology and Z-N staining. Int J Res Med Sci 2019;7:2985-8.